17C shows an array of heater elements **1008** compatible with the cartridge layout of FIG. **17**A.

[0142] The various embodiments shown in FIGS. **12**-**17**C are compatible with liquid dispensers, receiving bays, and detectors that are configured differently from the other specific examples described herein.

[0143] During the design and manufacture of highly multiplexed cartridges, photolithographic processing steps such as etching, hole drilling/photo-chemical drilling/sand-blasting/ion-milling processes should be optimized to give well defined holes and microchannel pattern. Proper distances between channels should be identified and maintained to obtain good bonding between the microchannel substate and the heat conducting substrate layer. In particular, it is desirable that minimal distances are maintained between pairs of adjacent microchannels to promote, reliable bonding of the laminate in between the channels.

[0144] The fabrication by injection molding of these complicated microfluidic structures having multiple channels and multiple inlet holes entails proper consideration of dimensional repeatability of these structures over multiple shots from the injection molding master pattern. Proper consideration is also attached to the placement of ejector pins to push out the structure from the mold without causing warp, bend or stretching of it. For example, impression of the ejector pins on the microfluidic substrate should not sink into the substrate thereby preventing planarity of the surface of the cartridge. The accurate placement of various inlet holes (such as sample inlet holes, valve inlet holes and vent holes) relative to adjacent microfluidic channels is also important because the presence of these holes can cause knit-lines to form that might cause unintended leak from a hole to a microchannel. Highly multiplexed microfluidic substrates may be fabricated in other materials such as glass, silicon.

[0145] The size of the substrate relative to the number of holes is also factor during fabrication because it is easy to make a substrate having just a simple microfluidic network with a few holes (maybe fewer than 10 holes) and a few microchannels, but making a substrate having over 24, or over 48, or over 72 holes, etc., is more difficult.

Microfluidic Networks

[0146] Particular components of exemplary microfluidic networks are further described herein.

[0147] Channels of a microfluidic network in a lane of cartridge typically have at least one sub-millimeter cross-sectional dimension. For example, channels of such a network may have a width and/or a depth of about 1 mm or less (e.g., about 750 microns or less, about 500 microns, or less, about 250 microns or less).

[0148] FIG. **18** shows a plan view of a representative microfluidic circuit found in one lane of a multi-lane cartridge such as shown in FIGS. **10**A and **10**B. It would be understood by one skilled in the art that other configurations of microfluidic network would be consistent with the function of the cartridges and apparatus described herein. In operation of the cartridge, in sequence, sample is introduced through liquid inlet **202**, optionally flows into a bubble removal vent channel **208** (which permits adventitious air bubbles introduced into the sample during entry, to escape), and continues along a channel **216**. Typically, when using a robotic dispenser of liquid sample, the volume is dispensed accurately enough that formation of bubbles is not a significant problem, and the presence of vent channel **208** is not necessary. Thus, in certain embodiments, the bubble removal vent channel **208** is not present and sample flows directly into channel **216**. Throughout the operation of cartridge **200**, the fluid is manipulated as a microdroplet (not shown in the FIGs). Valves **204** and **206** are initially both open, so that a microdroplet of sample-containing fluid can be pumped into PCR reactor channel **210** from inlet hole **202** under influence of force from the sample injection operation. Upon initiating of processing, the detector present on top of the PCR reactor **210** checks for the presence of liquid in the PCR channel, and then valves **204** and **206** are closed to isolate the PCR reaction mix from the outside. In one embodiment, the checking of the presence of liquid in the PCR channel is by measuring the heat ramp rate, such as by one or more temperature sensors in the heating unit. A channel with liquid absent will heat up faster than one in which, e.g., a sample, is present.

[0149] Both valves **204** and **206** are closed prior to thermocycling to prevent or reduce any evaporation of liquid, bubble generation, or movement of fluid from the PCR reactor. End vent **214** is configured to prevent a user from introducing an excess amount of liquid into the microfluidic cartridge, as well as playing a role of containing any sample from spilling over to unintended parts of the cartridge. A user may input sample volumes as small as an amount to fill the region from the bubble removal vent (if present) to the middle of the microreactor, or up to valve **204** or beyond valve **204**. The use of microvalves prevents both loss of liquid or vapor thereby enabling even a partially filled reactor to successfully complete a PCR thermocycling reaction.

[0150] The reactor **210** is a microfluidic channel that is heated through a series of cycles to carry out amplification of nucleotides in the sample, as further described herein, and according to amplification protocols known to those of ordinary skill in the art. The inside walls of the channel in the PCR reactor are typically made very smooth and polished to a shiny finish (for example, using a polish selected from SPI A**1**, SPI A**2**, SPI A**3**, SPI B**1**, or SPI B**2**) during manufacture. This is in order to minimize any microscopic quantities of air trapped in the surface of the PCR channel, which would causing bubbling during the thermocycling steps. The presence of bubbles especially in the detection region of the PCR channel could also cause a false or inaccurate reading while monitoring progress of the PCR. Additionally, the PCR channel can be made shallow such that the temperature gradient across the depth of the channel is minimized.

[0151] The region of the cartridge **212** above PCR reactor **210** is a thinned down section to reduce thermal mass and autofluorescence from plastic in the cartridge. It permits a detector to more reliably monitor progress of the reaction and also to detect fluorescence from a probe that binds to a quantity of amplified nucleotide. Exemplary probes are further described herein. The region **212** can be made of thinner material than the rest of the cartridge so as to permit the PCR channel to be more responsive to a heating cycle (for example, to rapidly heat and cool between temperatures appropriate for denaturing and annealing steps), and so as to reduce glare, autofluorescence, and undue absorption of fluorescence.

[0152] After PCR has been carried out on a sample, and presence or absence of a polynucleotide of interest has been determined, it is preferred that the amplified sample remains in the cartridge and that the cartridge is either used again (if one or more lanes remain unused), or disposed of. Should a user wish to run a post amplification analysis, such as gel electrophoresis, the user may pierce a hole through the lami-